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Autophagic Punctum

Growth factor signaling permits hypoxia-induced autophagy by a HIF1 α -dependent, BNIP3/3L-independent transcriptional program in human cancer cells

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Several recent reports have demonstrated that autophagy is induced in response to hypoxia in cultured cells. However, the mechanism and consequence of hypoxia-induced autophagy remains unclear as there is no consensus between these studies. In our recent report we show that, in human cancer cells, hypoxia cooperates with growth factor signaling to facilitate a HIF1 α -driven transcriptional response that promotes autophagy. Here we summarize these findings and set them in context of the findings of other groups, concluding that there are likely multiple routes to different forms of autophagy that serve different purposes downstream of hypoxia, depending upon the degree of stress and cellular context.

Screening of a kinase RNAi library in cultured *Drosophila* S2 cells was performed in our laboratory to identify novel regulators of autophagy. The rationale for this was that identification of pathways that selectively signal to autophagy in some contexts may allow us to ultimately dissect the different roles of autophagy in effecting diverse outcomes downstream of different stress stimuli. We identified *Pvr*, the *Drosophila* orthologue of the human PDGF receptor and VEGF receptor proteins, as a mediator of hypoxia-induced autophagy. Using pharmacological inhibitors and RNAi approaches we show that this role is evolutionarily conserved in a variety of human cancer cell lines, specifically implicating PDGF family receptors in the control of autophagy, the two receptors expressed and involved in our main model cell line being PDGFR β and FMS.

The requirement for growth factor signaling is selective for hypoxia-induced autophagy as it is not required for amino acid

starvation-induced or glucose deprivation-induced autophagy. Interestingly, this paradigm for growth factor control of hypoxia response-associated autophagy contrasts with that in some other cell types where a starvation response-associated autophagy may be repressed by growth factors, as these promote the uptake of nutrients from the extracellular milieu. The growth factor signaling to autophagy is autocrine in nature in our system, implicating the partial growth factor self-sufficiency of tumor cells in mediating autophagy. In tumors in vivo it is possible that this signaling might not only be provided by the tumor cells but may also operate in a paracrine fashion from stromal cell types such as myofibroblasts. This remains to be tested.

Mechanistically, we found that hypoxia-induced autophagy in human tumor cells occurs via HIF1 α -dependent transcriptional activity. However, this is independent of the HIF1 α targets that are required for mitochondrial autophagy, *BNIP3* and *BNIP3L*, despite HIF1 α -mediated upregulation of transcripts from these genes. This fits with the observation that, in human tumor cells, mitochondria are not degraded by the induced autophagy, whereas the p62/SQSTM1 adaptor protein for ubiquitinated autophagy cargoes is. However, in a primary cell type, mouse embryonic fibroblast, mitochondria are degraded by hypoxia-induced autophagy. Thus HIF1 α target genes may mediate different forms of autophagy, depending upon cellular context. Perhaps, in tumor cells, a higher glycolytic rate and lower dependence upon oxidative phosphorylation might mean that mitochondrial downregulation is less of a necessary adaptation to lower oxygen than in primary cells. Interestingly, our data imply that *BNIP3/3L* upregulation by hypoxia may not be sufficient for mitophagy but may cooperate with other cellular signals to mark mitochondria for autophagic degradation.

Growth factor signaling inhibition results in the increased lability of HIF1 α at a given oxygen tension, reducing its activity. This inhibition of HIF1 α activity selectively compromises the upregulation of a number of hypoxic target genes, but by no means all. This demonstrates that some functions of HIF1 α -mediated cellular reprogramming and adaptation to hypoxia are robust in nature, whereas others, including induction of autophagy, are

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sensitive to, and thus integrate signals from, other environmental conditions, such as the presence of growth factor. What other HIF1 α target genes, if not *BNIP3* and *BNIP3L*, might then mediate hypoxia-induced, nonmitochondrial autophagy? This is an active area of investigation within the laboratory. However, it is interesting to note that within the sets of genes that require growth factor signaling for regulation upon hypoxia are several potential regulators of vesicle trafficking processes, such as *RIN2*, a predicted Rab5GEF, or *SYTL2*, a potential interacting partner of Rab27. It is possible these play a role in mediating hypoxia-induced autophagy.

Finally, we show that the hypoxia-induced autophagy in human cancer cells is an adaptive response mediating cell survival. This suggests that one function of the autocrine growth factor signaling that is often observed in human cancer cells is to mediate tolerance of fluctuating oxygen tensions in growing tumors, at least partially via permitting hypoxia-induced autophagy. We have not specifically explored the consequence of cycles of hypoxia and reoxygenation, which may more specifically mimic the *in vivo* situation in evolving tumors. This is a research question that should be of interest in the future. Other reports have also demonstrated a prosurvival role for autophagy downstream of hypoxia, although the mechanism of this is only clear in the instance of hypoxia-induced mitophagy in primary cells (see above), where clearance of mitochondria is required to buffer cells against reactive oxygen species accumulation. We speculate, based on recent findings in the field, that autophagy may exert a negative regulatory effect on the ER-stress response; prolonged metabolic stress (i.e., hypoxia and nutrient deprivation) is known to induce this response, specifically in cells where autophagy is compromised.

Overall, we suggest that further investigation of the mechanisms by which hypoxia induces autophagy (Fig. 1) will assist understanding of how signaling pathways altered in cancer affect tumorigenesis via this route. Ultimately this may allow for therapeutic targeting of autophagy in cells within the hypoxic regions of tumors, without effects on the homeostatic, beneficial forms of autophagy in normal cells. Furthermore, some of these future findings will likely also have relevance to the pathology of other conditions where hypoxia is a factor such as cardiac ischemia/reperfusion injury.

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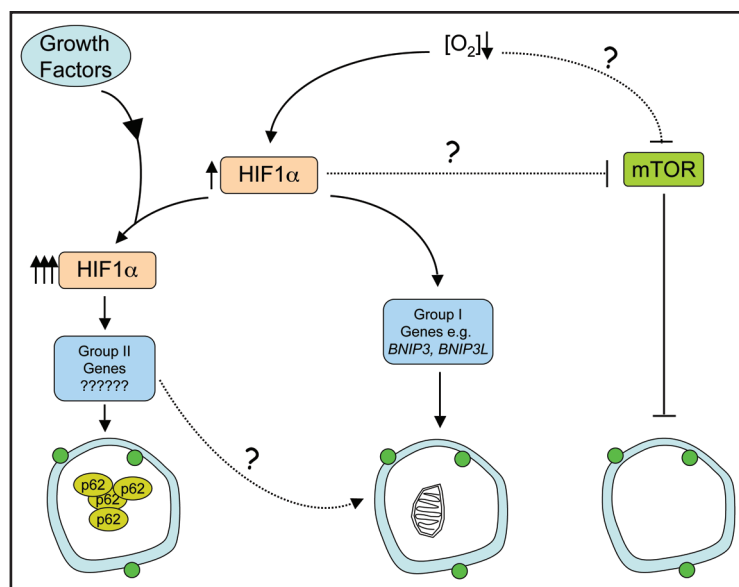


Figure 1. Model of multiple pathways to autophagy from hypoxia. Stabilization of HIF1 α upon hypoxia leads to transcriptional upregulation of target genes. Growth factor signaling enhances the transcriptional activity of HIF1 α by increasing its half-life. This enables HIF1 α to drive transcription of genes promoting macroautophagy of cytosolic targets such as p62/SQSTM1. HIF1 α can also drive transcription of *BNIP3* and *BNIP3L/Nix*, which may act as adaptors to mark mitochondria for inclusion in autophagosomes, although they are not necessarily sufficient for mitophagy per se. It remains to be tested whether the broader growth factor-driven transcriptional program downstream of HIF1 α is implicated in *BNIP3/3L*-mediated mitophagy. It also remains to be tested whether mTOR inhibition is a major signaling event upstream of autophagy at low ambient oxygen—either HIF1 α -dependent mTOR inhibition through *REDD1* transcription or HIF1 α -independent mTOR downregulation. Interestingly, very low oxygen (anoxia) inhibits mTOR and thus activates autophagy via activation of the energy-sensing AMP kinase. Group I genes are hypoxia-induced genes, the induction of which is dependent on HIF1 α . Group II genes are hypoxia-induced genes that are dependent on both HIF1 α and growth factor signaling.